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"STUDIES ON FUNGAL WILT PATHOGENS OF SELECTED SEEDS AND THEIR CONTROLLING STRATEGIES"

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Format for submission of Ph.D. Synopsis/ Work plan
For faculty of Science to be submitted in front of RRC

Abstract of proposed work plan/problem:

1. Introduction

2. Review of Literature and Development in the subject:

3. Objectives of Research/Proposed Hypothesis:

4. Methodology to be adopted:

5. Importance of study/Society application:

   - International status
   - National Status
   - Significance of the study

6. Proposed work Plan/Formulation and Structure of Study:

   - Year-wise Plan of work and targets to be achieved

7. References

Signature
Research Scholar

Signature
Research Supervisor /Guide/Co-Guide

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Introduction:

Fungi

Fungi are the most abundant group of plant pathogens. There are thousands of fungi capable of causing plant diseases. Fungi enter plants either through wounds or natural openings or by direct penetration. The 'body' of a fungus is composed of filament-like threads called 'hyphae.' Masses of hyphae are called 'mycelia.' Spores or mycelial fragments (these infective units are referred to as 'propagules') can be spread via wind, water, soil, insects, animals, and humans. Fungi reproduce via spores, which can be produced sexually or asexually. Spores vary in color, shape, size, and function, and this variation can often be used by diagnosticians as a means to identify pathogens.

Some fungi produce spores within sexual fruiting structures (ascocarps, pustules, mushrooms) or asexual fruiting structures. These spore types are more sensitive to environmental conditions than enclosed spores. Once a fungal spore makes contact with a plant surface, it germinates, much like a seed, as long as conditions are favorable for the pathogen. Hyphae emerge from spores and are capable of infecting plants via natural plant openings (such as stomata), through wounds, or by direct penetration of the plant epidermis. Upon infection, fungi utilize nutrients from their hosts. Many fungi produce additional spores as they grow, which aids in the spread of the pathogen.

Pathogens A plant disease is any physiological or structural abnormality that is caused by a living organism. Organisms that cause disease are referred to as 'pathogens,' and affected plants are referred to as **'hosts.**' Many organisms rely on other species for sources of nutrients or as a means of survival, but are not always harmful to the host.

Host plant genetic makeup determines its susceptibility to disease. This susceptibility depends upon various physical and biochemical factors within the plant.

Plants may also produce biochemical compounds that limit or prevent colonization or infection. Growth stage and ability to determine pathogens can also impact plant susceptibility to disease.

Environment plays an important role in disease development. Pathogens generally require specific environmental conditions for infection and spread. Most plant pathogens require high humidity and moderate temperatures. Other pathogens, such as

bacteria and water molds, require surface water for spread. In some disease cycles, environmental conditions influence the development of symptoms.

Common symptoms caused by fungi:

Leaf spots, wilts, blights, cankers, fruit rots, and dieback. Fungi cause a wide range of diseases including Septoria .leaf spot, powdery mildew, cedar-apple rust, and Armillaria root rot. **Powdery mildew** is one example of a disease in which fungal mycelia is visible.

Wilts: It is the drying or wilting of the entire plant. The leaves and other green or succulent parts lose their turgidity, becomes flaccid & droop. The effect is usually seen in some of the leaves. A **wilt disease** is any number of diseases that affect the vascular system of plants. Attacks by fungi, bacteria, and nematodes can cause rapid killing of plants, large tree branches or even entire trees. Plants have viruses too.

Pathogens that cause wilting diseases invade the vascular vessels and cause the xylem to fail to transport water to the foliage, thus causing wilting of stems and leaves.

Some wilt affected plants are-

Pigeonpea [*Cajanus cajan* (L.)]:

(*Cajanus cajan*) is one of the important leguminous crop of the tropics and subtropics .It is a major pulse crop of India. Dry dehulled split seeds are used as "*Dal"* for preparing "*Varan"*, "*Dal*" or *"Sambhar*". Green seeds as vegetable, crushed dry seed as animal feed, green leaves as fodder and stem as fuel wood are being used.

Causal Organism: *Fusarium udum* Butler var. *cajani.*

Symptoms: Yellowing followed by drying of leaves and finally death of few branches or of entire plant are the conspicuous symptoms manifested during flowering or grain development.

Infection of the plants in early stage lead to infection of roots, stem cortex and reaching up to vascular bundles where the pathogen multiplies and blocks water and nutrient flow to upper region which lead to yellowing, drying and finally death of the plant.

Production:

Pigeonpea seeds have 21% protein. Most of the world area under Pigeonpea is occupied by India with 78.6 % of production of Pigeonpea by India alone. Major Pigeonpea growing states of India include Maharashtra, Karnataka, Andhra Pradesh, Uttar Pradesh, Madhya Pradesh and Gujarat. Though Maharashtra ranks first in area and production, productivity of Maharashtra is only 663 kg/ha.

Losses:

Major cause of low productivity is the losses due to diseases. Among diseases wilt and sterility mosaic are important. Recent surveys have indicated that major losses in the Pigeonpea are due to wilt disease which is caused by *Fusarium udum* Butler var. *cajani.*

Losses ranging between 0.2 to 100 % have been estimated from India (Butler 1906, Patil 1984, Kannaiyan *et al.,* 1984 and Gade 2002).

Kannaiyan *et al.,* (1984) recorded maximum mean losses to the tune of 22 % from Maharashtra.

Economic Importance:

It is an important source of protein in the cereal based vegetarian diet. It is largely consumed in the form of 'Dal'. Pigeonpea plays an important role in farming system because it fixes the atmospheric nitrogen in the soil.

Control

The crop is affected by more than hundred pathogens. These includes fungi, bacteria, viruses, nematodes and mycoplasma like organisms. Major diseases of Pigeonpea are, wilt (*Fusarium udum* Butler), among these diseases, wilt caused by *Fusarium udum* Bulter, is the most destructive soil and seed born disease of Pigeonpea.

(*Cajanus cajan*) is one of the important leguminous crop of the tropics and subtropics and is infected by the wilt pathogen *Neocosmospora vasinfecta* in addition to *Fusari*e many workers have been reported that the wilt in Pigeonpea was caused by *Necosmospora vasinfecta* it was proved that the fungicides viz. carbendazim, combinations of carbendazim+mancozeb and thiram+carbendazim as well as bioagent, *T. herzianum* were effective against *Neocosmospora* wilt of pigeon pea under *in vitro* condition.

2. Soybean: [*Glycin max***]**

Soybean also called soja bean or soya bean, annual legume of the pea family fabaceae and its edible seed. It is economically the most important bean in the world, providing vegetable proteins for millions of people and ingredients of hundreds of chemical products.

Causal organism:

Isolated from diseased soybean plants were the species *Fusarium avenaceum, F. equiseti, F. oxysporum* and *F. poae.*

Symptoms:

Fusarium wilt and necrosis of root and lower stem of soybean, leaf chlorosis, wilt of the apical portion of the plant, necrosis of the root and lower stem, and wilting of the whole plant. The pods are often poorly developed. The seeds may be smaller and lighter in the weight and infected, as well.

Production:

United States is world's largest Soybean producer with88 million tonnes production every year. U.S is followed by Brazil, Argentina. With 12 million tonnes of production; Soyabean is one of the fastest growing crops in India. Top three soyabean growing states are Madhya Pradesh, Maharashtra and Rasjasthan.

Losses:

The *Fusarium* wilt and necrosis of root and lower of soybean (abbreviated to FWNS) is an important disease in many countries. It can cause great damage, as it may reduce the average yield of soybean by up to 59% (Sinckla-ir and Backman, 1989).

Soybean diseases can be a significant economic factor in Arkansas. On average, diseases reduce yields in the state by an estimated 10%, although in individual fields and with certain diseases, losses may be much higher.

Economic importance:

It is the richest and cheapest sources of protein. Seed contains 70% oil, 63% meal, 50% protein. Soya bean contains no starch they are good source of protein for diabetics.

Control:

A fungicide seed treatment can provide a healthy start for seedlings. Multiple fungal pests can impact soybean seeds and seedlings immediately after planting,

and EverGol® Energy SB can protect against pests causing the most damage such as *Rhizoctonia solani, Fusarium spp and Pythium spp.*

Bayer offers Stratego® YLD, a fungicide featuring triazole technology combined with strobilurin chemistry for soybeans. Offering two modes of action, it provides both preventive and curative benefits and systemic movement to provide broad-spectrum, long-lasting disease control and higher yield potential.

3. Tomato:

Tomato (*Lycopersicon esculentum* Mill.) belongs to the family Solanaceae. It is one of the world's major vegetables. Tomato is a major vegetable crop, it is grown in practically every country of the world. In outdoors fields, greenhouses and nethouses.

Causal organism:

Fusarium oxysporum f.sp. *lycopersici*

(*Found* worldwide, more prevalent in warmer climates.)

Symptoms:

Yellowing of the foliage, Infected leaves later show downward curling, followed browning and drying. Vascular browning in infected stems and large leaf petioles. Affected plants and their root systems are stunted. The degree of stunting depends upon time of root infection. Plants infected when they are young will be more severely stunted than plants infected at a later stage.

Production:

A total area and production of 4.4 million ha and 115 million metric tons, respectively. As it is a relatively short duration crop and gives a high yield, it is economically attractive and the area under cultivation is increasing daily.

Losses:

Yield losses of 100% are common, particularly when tomatoes are infected early in development. The average global crop losses of all diseases combined was approximately 12.8% of the potential production but tomato alone was subjected to 21.8% loss. In Ethiopia the disease caused 100% crop loss on unimproved local cultivar, and 67.1% on susceptible cultivar.

Economic importance: It is good source of vitamins A and C. vitamin A important for bone growth, cell division and differentiation, maintaining surface linings of eyes, regulation of immune system. Vitamin C important in forming collagen protein that gives structure to bones, cartilage, muscles and blood vessels. Lycopene is very

powerful antioxidant which can help prevent the development of many forms of cancer.

Control: The spores of this tomato plant disease live in the soil and can survive for many years. They're spread by equipment, water, plant debris, and even people and animals. The best method of prevention is to plant resistant varieties if you've had trouble with Fusarium wilt in the past. Also disinfect tomato cages and stakes with a 10% bleach solution at the end of every season.

Manage: Soil solarization can help kill fungal spores in the top few inches of soil, and crop rotation is key. There are also several biological fungicidal drenches that can be applied to soil (look for one based on the bacteria *Streptomyces griseoviridis* called MycoStop® or a granular one based on the fungus Trichoderma virens called Soil Guard®). These products may help prevent the infection from colonizing the roots of future crops.

Mycostop is a biological fungicide that will safely protect crops against wilt caused by *Fusarium*. Approved for use in organic crop production, it can be applied as a soil spray or drench (1-2 gm/ 100 sq. ft.) to seedlings, ornamentals and vegetables. Apply sufficient water during application to move Mycostop into the root zone.

4. Brinjal: (*Solanum melongena* L.)

It is one of the most common, and highly productive vegetables grown globally and cultivated widely in India. It is annual crop cultivated all over India. Fruits are available throughout the year.

Causal organism: *Verticillium* wilt (*Verticillium dahliae*), *fusarium* wilt (*Fusarium oxysporum* f. sp. *melongenae*)

Bacterial wilt

(*Ralstonia solanacearum*) (Kalloo and Berg, 1993) and (Sihachakr *et al.*, 1994).

Symptoms:

Fusarium spp. is the most destructive pathogen that causes wilt in brinjal. This pathogen blocks the xylem transport system and causes severe wilting and death of brinjal plants.

Fusarium wilt symptoms also include drooping, yellowing, wilting, and dying of the lower leaves, often on one side of the plant ,browning of the vascular system .Plant growth is stunted and, under warm conditions, the plant may die.

Production:

Eggplant is the second most important vegetable crop next to potato in Bangladesh in respect of acreage and production (BBS, 2005). It is cultivated a commercially throughout the tropical and subtropical region of the world. It is also popular in other countries like Balkan area, France, Indonesia, Italy, Japan, Mediterranean, Turkey and United states (Bose and Som, 1986).

Losses:

The average yield is 6.0 t/ha in 2003-04 (BBS, 2005). The yield potential of eggplant is low in Bangladesh compared to other countries. Of many reasons for high price of eggplant, lower production rate is important. Incidence of insect pests and diseases greatly hampered the production of eggplant. This crop suffers from the various diseases; about 13 different diseases so far recorded in Bangladesh (Das et al. 2000; Khan et al. 1998 and Rashid, 2000). Among those diseases wilt of eggplant has been treated as one of the major constrains in eggplant cultivation in the country (Ali, 1993).

Economic importance: It is good source of calcium, phosphorus, iron, vitamins particularly B group. Green leaves and dark purple Brinjal are main source of vitamin C. It is reported to stimulate the intrapeptic metabolism of blood cholesterol.

5. Gram: *Cicer arietinum* (chickpea).

Chickpea**,** (*Cicer arietinum*), also called garbanzo bean or Bengal gram, annual plant of the pea family (Fabaceae), widely grown for its nutritious seeds. Chickpeas are an important food plant in India, Africa, and Central and South America. The seeds are high in fibre and protein and are a good source of iron, phosphorus, and folic acid.

Causal organism: Fusarium wilt of chickpea

Fusarium oxysporum f.sp. ciceris

Symptoms:

Leaves – wilting, yellowed or dead, whole plant - plant dead; dieback.

Chickpea foliage develops a greyish-green chlorosis, typically affecting lower leaves first and extending up the plant. Leaves eventually take on a dull-yellow colour, wilt and the plant collapses and dies. In some cases there may be leaf vein clearing before

wilt begins. Internally, the xylem tissues stain dark-brown to almost black. Wilting may initially affect only one side of the plant.

Production:

Cicer arietinum L. (chickpea) is the second most cultivated leguminous crop with 13.1 million tons per annum production, commonly grown on an estimated 13.5 million hectares of land in tropical, subtropical, temperate and semi-arid areas, worldwide.

India is amongst the chief producers of chickpea, having 8.63 mha area under cultivation, 7.85 mt annum −1 production with an average yield of 900 kg per hectare (CIME, 2010; FAOSTAT, 2015; Muehlbauer and Sarker, 2017).

Economic importance: It constitutes 20-30% protein, 40-59% carbohydrate, 3% fibre, 3-6% oil, 4% ash, and is a good source of absorbable ions like Ca, P, Mg, Fe, K and essential B vitamins (Ibrikci et al., 2003).Green pods used are used as vegetable. Haulm is used as fodder, Excellent green manure as it is easily decomposed when incorporated (Biomass has 1.5% N) , Seed contains 25% protein, 1.15% fat and 62.6% carbohydrate , Sprouted seeds which are rich in vitamins are consumed as salad , Flour is used in cakes. Starch is used in making noodles, being short duration fits well in many intensive crop rotations.

Control:

The use of germplasm resistant to *F. oxysporum* f.sp. *ciceris* is the primary means of disease control. Losses may also be reduced by fungicide applications (Shugha et al., 1995), maintaining high levels of organic matter in the soil and avoiding very early planting (Padwick and Bhagwagar, 1943). As the fungus is seedborne, it is important to use seed from disease-free fields. Deep ploughing during summer, removal of host debris from the field and excluding chickpea from the crop rotations in infested fields for at least 3 years can reduce the level of inoculum. Soil solarization can effectively eradicate inoculum in soil. Systematic examination of seed production fields and removal of undesirable plants from the seed fields from time to time are essential in maintaining varietal purity and also protecting the seed crop from seedborne diseases. Seed cleaners and colour sorters can be used to sort out diseased or damaged seed

resulting in better germination rates and a healthier crop stand.Good control can be obtained with a combination of treatments, such as combining fungicides with biological controls and deep ploughing.

Control through Plant extract:

The antifungal effect of aqueous extracts of four plant species;
Azadaracta indica A. Juss., *Datura metel* L. var. *quinquecuspida* Torr, *Ocimum sanctum* L. and *Parthenium hysterophorus* L. was observed *in vitro* study.
Leaf extract of *Azadiracta indica* at 100% conc. completely inhibited germination of pathogen spores.

According to Sharma and Kumar, extract of three weed plants, namely, *Capparis decidua, Lantana camara* and *Tridax procumbens,* show antifungal property against *Fusarium oxysporum*. Acetone extracts of *Datura stramonium* have been reported to have antifungal activity against several fungi including *Fusarium oxysporum*.

In botanical *Solanum nigrum* L., *Tagetes erecta, Polyalthia longifolia, Parthenium hysterophorus* L., *Zingiber officinale* was also most effective against *F. udam*.
All the plant extracts, *Calotropis procera, Eucalyptus globulus, Jatropha multifida, Azadirachta indica, Allium sativum* tested, except *Calotropis procera*, were significantly superior in reducing wilt incidence in chickpea.

Some local medicinal plants (*Anacyclus valentinus* L., *Artemisia herba* alba Asso, *Eucalyptus* sp, *Inula viscosa* (L.) Aiton, *Laurus nobilis* L., *Mentha pepirita* L., *Rosmarinus officinalis* L., *Salvia officinalis* L., *Tetraclinis articulata* and *Thymus vulgaris* L.) show antifungal property against F. *oxysporum* f.sp. *lentis.*

Allium sativum extract, *Azadirachta indica* leaf extract, *Zingiber officinale* Extract, *Calatropis procera* leaf extract, *Moringa oleifera* leaf extract and *Parthenium hysterophorus* L. leaf extract show fungitoxic effect against F. *oxysporum* f.sp. *pisi.*

Biological and Chemical control

Control of plant disease is a pressing need for agriculture in the 21st century. The increasing demand for a steady, healthy food supply by a burgeoning human population will require controlling diseases that reduce crop yield. Furthermore, increased pressure for food production will intensify the demands on agricultural production systems, which in turn, may increase disease pressure on crop plants.

Current practices for controlling plant disease are based largely on genetic resistance in the host plant, management of the plant and its environment, and synthetic pesticides. There is a demand for new methods to supplement existing disease control strategies to achieve better disease control. Moreover, alternatives too many of the synthetic pesticides currently in use are needed. Many of the synthetic chemicals may lose their usefulness due to revised safety regulations or development of resistance in pathogen populations. Thus, there is a need for new solutions to plant disease problems that provide effective control while minimizing negative consequences for human health and the environment.

Biological control, using microorganisms to suppress plant disease, offers a powerful alternative to the use of synthetic chemicals. The rich diversity of the microbial world provides a seemingly endless resource for this purpose. Increasing the abundance of a particular strain in the vicinity of a plant can suppress disease without producing lasting effects on the rest of the microbial community or other organisms in the ecosystem Biological control is also likely to be more robust than disease control that is based on synthetic chemicals. The complexity of the organismal interactions, the involvement of numerous mechanisms of disease suppression by a single microorganism, and the adaptedness of most biocontrol agents to the environment in which they are used all contribute to the belief that biocontrol will be more durable than synthetic chemicals.

Review of literature:

Fungus wilt Pathogen

(*Cajanus cajan*) is one of the important leguminous crop of the tropics and subtropics and is infected by the wilt pathogen *Neocosmospora vasinfecta* in addition to *Fusari*e many workers have been reported that the wilt in Pigeonpea was caused by *Necosmospora vasinfecta* (Anamorph, *Acremonium* spp.).

Sarojini reported the wilt of Pigeonpea was caused by *N. vasinfecta* and observed the similar symptoms to those incited by *F. udum* and wilt incidence was observed in the range of 62-100%.
Giri (Unpublished data) noted the association of *N. vasinfecta*, while studying the pathogenic variability among the isolates of *F. udum* from different locations of Vidarbha region.

Vishwa *et al.* reported the wilt of Pigeonpea caused by *N. vasinfecta* from experimental as well as farmers filed. Maximum 72.4% wilting was recorded at 60 days after sowing which indicates the strong virulence of pathogen.

Tomato powdery mildew caused by *Oidium neolycopersicum* has started to cause severe epidemics on tomato during the last 15 years (Jones et al., 2001).

Heavy infections were first recorded in Western Europe and during the 1980s it was reported for the first time in North America (Mieslerová & Lebeda, 1999). It is a dangerous pathogen, which spread through temperate areas of the world. The host range of the pathogen is broad and it is reported to attack over 60 species in 13 plant families, particularly members of *Solanaceae* and *Curcubitaceae*. (Jones et al., 2001).

Symptoms include powdery white lesions on the adaxial leaves surface and on all other aerial plant parts except on the fruits. The fruits are not directly affected but impaired photosynthesis and premature senescence reduces fruit size andnutritional quality leading to diminished yields (Mieslerov'a & Lebeda, 1999).

The *Fusarium* wilt and necrosis of root and lower of soybean (abbreviated to FWNS) is an important disease in many countries. It can cause great damage, as it may reduce the average yield of soybean by up to 59% (Sincla-ir and Backman, 1989).

The fusariosis of soybean was first recorded in1917 in the U.S. (Cronewell, 1917) and has since been reported in many parts of the world (Sinclair and Backman, 1989). In our country, the disease was first observed and described in 1964 by Aaimovia (1988), and later by Tosia et al. (1986) as well.

In Serbia and Montenegro, the *Fusarium* wilt and necrosis of root and lower stem of soybean is present in some years to a greater or lesser extent. The FWNS was first observed in our country 1964 (Aaimaovia, 1988) and the causal organism was identified as *Fusarium* sp.

A disease with new symptomatology was observed on several pomegranate plants in pomegranate (Research orchard of Mahatma Phule Agriculture University, Rahuri, India). The disease symptoms include initial yellowing and drooping of plant leaves on one or two branches, followed by leaf fall and wilting of the affected branches. Subsequently, the same types of symptoms appear on other branches leading to its death. The entire plant wilts within a period of 2-3 months. There is no recovery of the wilted plant or wilt branches.

Fusarium wilt of banana, popularly known as Panama disease, is a lethal fungal disease caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*). The fungus enters the plant through the roots and colonizes the xylem vessels thereby blocking the flow of water and nutrients (Ploetz and Churchill, 2011).

Fusarium wilt or Panama disease of banana produces two types of external symptoms: “yellow leaf syndrome” and “green leaf syndrome” (Stover, 1962; Pérez-Vicente, 2004).

Medicinal plants

According to Sharma and Kumar, extract of three weed plants, namely, *Capparis decidua, Lantana camara* and *Tridax procumbens,* show antifungal property against *Fusarium oxysporum.*

In a search for plant extracts with potent antifungal activity against plant pathogenic fungi, the methanol extracts of different medical plants effectively controlled the development of plant diseases caused by *Macrophomina phaseolina*.

The methanolic extracts of forty nine plants hexane, chloroform and methanolic extracts were used for the study, among them methanolic extracts of forty eight medicinal plants exhibited varying degrees of inhibition activity against *M. phaseolina*, extracts of *Tephrosia pumila* have no activity.

Extracts of *Avicenia officinales, Cleome viscose, Grewia arborea, Hyptis sueolences, Ocimum sanctum, Peltophorum pterophorus, Sesbanian grandiflora, Terminalia chebula, Tephrosia pumila, Tephrosia villosa, Withania somnifera* showed high activity and *Terminalia chebula* having strong antifungal activity against *M. phaseolina* even at low concentrations. Chloroform extracts has very low antimicrobial activity whereas hexane has no antimicrobial properties. (Varaprasad Bobbarala et al. 2009).

Chemical control method:

Since the first use of fungicides in the 1800s, synthetic chemicals have provided much needed relief in the management of plant fungal disease in agricultural production.

Seed treatment method with some chemicals such as equivalent mixture of benomyl and thiram108, a combination of carbendazim + thiophanate (0.15 + 0.10%) 72 were found to be very effective in dropping the Fusarium wilt population.

It was also reported that application of boron (Bo), zinc (Zn) or manganese (Mn) and methyl bromide (CH3Br) to the soil drastically reduce the disease event of Fusarium wilt.

Lentil wilt is controlled by the seed treatment with thiram + pentachloronitrobenzene or thiram + carboxin. Antracol, Captan, Benlate, Topsin-M, Cobox, Dithane M-45,

Acrobat, Pentachloronitro-benzene (PCNB), Ridomil, Sancozeb, Vitavax, Daconil and Trimiltox-forte are sensitive against *oxysporum* f. sp. *ciceris* and studied on the basis of fungicides sensitivity @ 100 ppm using poison food technique .

Raxil Ultra (Tebuconazole), Topsin-M (Thiophanate Methyl), Score (Difenconazole), Derosil (Carbendazim), Hombre (Imidacloprid+Tebuconazole) and Divident Star (Metalaxy1M+Difenconazole) show fungicidal property against pea wilt.

Fungicides are frequently toxic to non-target organisms like earthworms, microbes and humans (genotoxicity) causing imbalances in the ecosystems.

3. Objectives of research:

1. Collection of infected material.

2. Isolation and purification of wilt pathogen from selected plant material.

3. Control of wilt pathogen through medicinal plant extract.

4. Control of wilt pathogen by chemical method.

5. Control of wilt pathogen by biological method.

6. Comparison between biological and chemical control method.

4. Methodology:

A] Isolation and purification of wilt pathogen from selected plant material.

1. Standard blotter paper
2. Agar plate method.

B] Biological control of wilt pathogen.

1. Isolated wilt pathogens controlled by biological method by using medicinal plant extract.
    - ❖ Phytochemical analysis of medicinal plants by HPTLC.

2. Fungal pathogen controlled by food poison technique.

    - ❖ Antifungal and antimicrobial activity of medicinal plants by using food poison technique.

C] Chemical control:

1. Different fungicides used for controlling such pathogen by food poison technique.

D] Comparison:

Statistical analysis of obtained data will be carried out by using the standard methods given by Panse, Sukhatme (1967) and Mungikar (1997). Statistically comparison carried out using two methods- ANNOVA and LSD.

5. Importance of the present study:

Control of plant disease is a pressing need for agriculture in the 21st century. The increasing demand for a steady, healthy food supply by a burgeoning human population will require controlling diseases that reduce crop yield. Furthermore, increased pressure for food production will intensify the demands on agricultural production systems, which in turn, may increase disease pressure on crop plants.

A] Assessment of seed borne Pathogens:

Seed Plays very important role in vegetables, crop cultivation. A high quality seed is an essential prerequisite for sustainable and profitable crop production. Absence of seed borne pathogens is one of the quality characters. High quality seed is not only need to be true to type, but also clean from contaminants, should have a high viability, and should be healthy(free from any pathogen).

B] Biological seed Treatment:

Biological seed treatment is usually very specialized and uses specific microorganisms that attack or interfere with specific pathogens or types of pathogens. The biological seed treatment involves use of biological organisms to control the pathogen located in the seed.

Biological is an attractive alternative for fungal pathogen control, since it neither causes environmental pollution nor induces pathogen resistance, which is usually a side effect of synthetic antifungal agents. Biological control is one of the viable, eco-friendly propositions, which can substantially minimize the disease (Cook in 1985). It has contributed significantly to reducing the environmental impacts of agricultural pest management, and in conserving indigenous species attacked by invasive insects.

Indiscriminate use of chemical pesticides all over the world deteriorated the soil mycoflora and damaged our environment beyond repairs. From last four decades reckless use of non-target pesticides to kill the pest, we lost lot of biodiversity and disturbed our ecosystem. Many efforts have been done to discover new antimicrobial compounds from various kinds of sources. Such pesticide activity has been explored in order to make available pesticides which are easily biodegradable, which can be locally produced, for farmers who cannot afford expensive synthetic pesticides.

Biological control:

Biological control has an extremely long association with agriculture, has played an integral role in crop integrated pest management programmes throughout the world. The increasing numbers of exotic, invasive pests resulting from international trade and travel that reach new geographic areas without their regulating populations of natural enemies present new opportunities for importation biological control to provide long-term cost-effective pest management.

- Biological control is less costly and cheaper than any other method.
- Biocontrol agents give protection to the crop throughout the crop period.
- They are highly effective against specific plant diseases, they do not cause toxicity to the plants.
- Biocontrol agents can eliminate pathogens from site of infection.

At the same time as the need to increase global food production has intensified (God fray et al., 2010), there is a more widespread understanding of potential environmental degradation associated with many modern agricultural practices (IAASTD, 2009). There is a growing realization in the environmental community that conservation of biological diversity and ecosystem function requires conservation projects to focus on developed lands, including agro-ecosystems, rather than just undeveloped lands to meet their goals (Brussaard et al., 2010). These trends combined with a developing appreciation for the value of ecological services may present opportunities for implementing conservation biological control on a larger scale. The increasing public demand for 'local' and organic foods may also be creating opportunities for increased implementation of biological control. As it has throughout the history of agriculture, biological control should continue to play an important role in integrated pest management of insect pests in cropping systems worldwide.

C] Antimicrobial activity of medicinal plants:

Plants that possess therapeutic properties on the animal or plant body are generally designated as Medicinal plants, with the development of microorganisms resistant to chemicals applied indiscriminately to crops, research has been done with the goal to search for alternative and safe forms of

agrochemical pest control without causing any damage to environment and to humans, maintaining the crop qualitatively and quantitatively.

Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Much work has been done on ethno-medicinal plants in India. Interest in a large number of traditional natural products has increased. Traditionally plants have been well exploited by man for the treatment of human diseases.

The objective to conduct this investigation is to find out the efficacy of bio agents on seed borne mycoflora to improve seed germination, shoot, root length and seedling vigour index.

6. Year wise plan of work:

Year I:

Isolation and purification of wilt pathogen from selected plant material is carried out. Slide preparation and scanning will be done as described earlier workers. Seed health testing of selected seeds will be accessed. Collection of literature on the concern work will be carried out.

Year II: In this year effectiveness of medicinal plants and fungal wilt pathogens will be measured. Reference collection would be done as required extent. Assembling of data obtained as a result of previous experiment will be done. Scanning of slide would be completed.

Year III:

Calculation, tables, plotting of graphs, micrographs, writing other paper work will be done.

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